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Original article

The effect of vitamin D on thyroid autoimmunity in euthyroid men with autoimmune thyroiditis and testosterone deficiency

Robert Krysiak[a,*], Karolina Kowalcze[b], Bogusław Okopień[a]

[a] Department of Internal Medicine and Clinical Pharmacology, Medical University of Silesia, Katowice, Poland
[b] Department of Paediatrics in Bytom, School of Health Sciences in Katowice, Medical University of Silesia, Katowice, Poland

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ABSTRACT

Background: Autoimmune (Hashimoto's thyroiditis) is characterized by a strong female preponderance, which may suggest that sex hormones have an impact on thyroid autoimmunity. The aim of this study was to investigate whether testosterone determines vitamin D action on thyroid antibody titers and thyroid function tests in men with autoimmune thyroiditis and low testosterone levels.
Methods: The study included 36 men with testosterone deficiency, 17 of whom had been treated for at least 26 weeks with oral testosterone undecanoate (120 mg daily). Because of coexistent euthyroid Hashimoto's thyroiditis, all participants were then treated with vitamin D (100 μg daily). Serum titers of thyroid peroxidase and thyroglobulin antibodies, serum levels of thyrotropin, free thyroid hormones, testosterone and 25-hydroxyvitamin D, as well as Jostel's thyrotropin index, SPINA-GT and SPINA-GD were assessed before vitamin D treatment and 26 weeks later.
Results: With the exception of testosterone levels, there were no significant differences between both study groups in serum hormone levels, antibody titers and thyroid function tests. All participants completed the study. In addition to increasing 25-hydroxyvitamin D levels, vitamin D increased SPINA-GT and reduced thyroid peroxidase and thyroglobulin antibody titers. In testosterone-treated men, vitamin D increased testosterone levels. Vitamin D did not affect serum levels of thyrotropin, free thyroid hormones, Jostel's thyrotropin index and SPINA-GD. Treatment-induced changes in thyroid antibody titers and SPINA-GT were more pronounced in testosterone-treated than testosterone-naïve men.
Conclusions: The obtained results suggest that the beneficial effect on thyroid autoimmunity and thyroid secretory function is stronger in men receiving testosterone therapy.

Introduction

Hashimoto's thyroiditis, also known as autoimmune thyroiditis, is the most prevalent organ-specific autoimmune disease, as well as the leading cause of primary hypothyroidism [1,2]. Autoimmune-mediated destruction of the thyroid gland leads to diffuse infiltration of the thyroid gland by specific B and T cells, the result of which is apoptosis of follicular epithelial cells and, at a later stage, their replacement by fibrous tissue [3,4]. Nearly all patients have high serum concentrations of antibodies directed against one or more thyroid antigens, particularly against thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) [1,2].

Numerous observational studies reported a link between the risk of development of autoimmune thyroiditis and vitamin D (cholecalciferol) deficiency. Patients with autoimmune thyroiditis were characterized by low 25-hydroxyvitamin D levels and this relationship persisted after adjustment for other predictive factors, such as sex, age and body mass index [5–7]. Serum levels of 25-hydroxyvitamin D were found to correlate with thyroid volume, duration of the disease as well as with TPOAb titers [8]. The increased risk of the development of Hashimoto's thyroiditis depended on TaqI and BsmI vitamin D receptor gene polymorphisms [9,10]. The role of vitamin D as a preventive agent in Hashimoto's thyroiditis was supported by the results of interventional studies. Exogenous vitamin D decreased thyroid antibody titers, both in patients with abnormally low 25-hydroxyvitamin D levels and in patients with 25-hydroxyvitamin D levels within the reference range [11,12]. Unfortunately, post-treatment values of thyroid antibody titers were still above the upper limit of normal.

Similarly to other disorders of the thyroid gland [1], autoimmune thyroiditis is characterized by a strong female

Abbreviations: CI, confidence interval; IU, international unit; SD, standard deviation; SPINA, structure parameter inference approach; TgAb, thyroglobulin antibodies; TPOAb, thyroid peroxidase antibodies.

* Corresponding author.
E-mail address: r.krysiak@interia.pl (R. Krysiak).

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preponderance, which suggests that sex hormones may have an impact on thyroid autoimmunity. The results of few studies conducted to date seem to be in line with this suggestion. The risk of development of autoimmune thyroiditis in men correlated with a value of the estradiol: testosterone ratio [13]. Impaired testicular function was observed in men with primary thyroid failure, most of whom had Hashimoto's thyroiditis [14]. Patients with Klinefelter syndrome, the most common genetic cause of primary hypogonadism in men, are more often diagnosed with autoimmune thyroiditis than their healthy counterparts [15]. The age of onset of autoimmune thyroiditis was found to be associated with the CAG repeat polymorphism of the androgen receptor, determining the strength of testosterone action [16].

No human studies have investigated the use of testosterone in subjects with Hashimoto's thyroiditis. However, in rats repeated doses of testosterone ester in oil and, to a lesser extent, also testosterone administration by implantation caused significant regression of chronic thyroiditis [17]. Moreover, in the obese strain of chickens, developing spontaneous autoimmune thyroiditis at several weeks of age, testosterone administration decreased thyroid infiltration by lymphocytes while castration of the control animals produced the opposite effect [18,19]. Therefore, the aim of our study was to find whether testosterone undecanoate modulates the effect on exogenous vitamin D on thyroid autoimmunity in men with autoimmune thyroid disease and testosterone deficiency.

Materials and methods

Patients

This case-control study included 36 men (40–70 years old) with untreated euthyroid Hashimoto's thyroiditis who had been previously diagnosed with testosterone deficiency. Seventeen of these men had been treated for at least 6 months with oral testosterone undecanoate (120 mg daily), while 19 men had not received any pharmacotherapy.[1] The inclusion criteria were as follows: (a) TPOAb titers above 100 U/mL, (b) reduced echogenicity of the thyroid parenchyma on thyroid ultrasonography (c) serum levels of thyrotropin and free thyroid hormones within the reference range (thyrotropin between 0.4 and 4.5 mU/L, free thyroxine between 10.1 and 21.1 pmol/L and free triiodothyronine between 2.2 and 6.5 pmol/L). Testosterone-naïve patients were selected among 35 men meeting the inclusion criteria. The purpose of this selection was to obtain two treatment arms well-matched for age, body mass index and thyroid antibody titers. To minimize the impact of seasonal fluctuations in 25-hydroxyvitamin D levels and thyroid antibody titers, 18 (8 testosterone-treated and 10 testosterone-naïve) men were recruited between December and January while the remaining 18 (9 testosterone-treated and 9 testosterone-naïve) men were enrolled in June or in July. Before enrolling patients, the study protocol was approved by the institutional review board and all participants provided written informed consent.

The exclusion criteria were as follows: positive serum antibodies against thyrotropin receptor, other autoimmune disorders, body mass index above 40 kg/m2, prostate cancer, prostate-specific antigen greater than 4 ng/mL (or prostate-specific antigen above 3 ng/mL in men at high risk of prostate cancer), severe lower urinary tract symptoms (the American Urological Association International Prostate Symptom Score above 19), breast cancer, impaired renal or liver function, congestive heart failure, myocardial infarction, stroke or coronary revascularization procedure in the past, hematocrit exceeding 50%, untreated obstructive sleep apnea, diabetes mellitus and poor patient compliance. Subjects taking any drugs (with the exception of testosterone) within 6 months before the beginning of the study were also excluded.

Study design

Throughout the entire period of the study (26 weeks), all patients were treated with vitamin D (100 μg [4000 IU] daily), administered in the morning (between 7 a.m. and 8 a.m.). Moreover, testosterone-treated patients continued treatment with the same dose of oral testosterone undecanoate as before the beginning of the study (120 mg daily), divided into three equal doses. For adequate absorption from the gastrointestinal tract, the participants were requested to take testosterone undecanoate with a meal containing dietary fat. Compliance was assessed once monthly during each visit by tablet counting.

Laboratory assays

Venous blood samples for laboratory assays were obtained between 8.00 and 9.00 a.m., following at least a 12-h overnight fasting in a quiet, temperature-controlled room (24–25 °C) at the beginning of the study and 26 weeks later. Serum levels of thyrotropin, free thyroxine, free triiodothyronine and testosterone, as well as serum titers of TPOAb and TgAb were assayed by direct chemiluminescence using acridinium ester technology (ADVIA Centaur XP Immunoassay System, Siemens Healthcare Diagnostics, Munich, Germany). Serum levels of 25-hydroxyvitamin D were detected by competitive immunoassay using and a multichannel automatic analyzer (Roche Cobas e 411, Mannheim, Germany) and commercially available Roche Diagnostic kits. All measurements were performed in duplicate.

The calculated parameters of thyroid homeostasis were determined with the use of SPINA-Thyr 4.0.1 for Mac Universal software. Jostel's thyrotropin index was assessed using the formula: ln [thyrotropin] + 0.1345 x free thyroxine [20]. SPINA-GD was a calculated as follows: β_{31} x (K_{M1} + free thyroxine) (1 + K_{30} x standard concentration of thyroxine-binding globulin) x free triiodothyronine/(α_{31} x free thyroxine) [21]. SPINA-GT was assessed using the following equation: β_T x (D_T + thyrotropin) x (1 + K_{41} x standard concentration of thyroxine-binding globulin + K_{42} x standard concentration of transthyretin x free thyroxine) / (α_T x thyrotropin) [22]. Constants used in the equations were as follows: $\beta_T = 1.1 \times 10^{-6}$/s, $D_T = 2.75$ mU/L, $K_{41} = 2 \times 10^{10}$ L/mol, standard concentration of thyroxine-binding globulin = 300 nmol/L, $K_{42} = 2 \times 10^{8}$ L/mol, standard concentration of transthyretin = 4.5 mmol/L, $\alpha_T = 0.1$/L, $\beta_{31} = 8 \times 10^{-6}$/s, $K_{M1} = 5 \times 10^{-7}$ mol/L, $K_{30} = 2 \times 10^{9}$ L/mol and $\alpha_{31} = 0.026$/L [21,22].

Statistical analysis

Data with skewed distributions (antibody titers, hormones and calculated parameters of thyroid homeostasis) were log-transformed to overcome heteroscedasticity. Between-group comparisons were made using Student's *t*-tests for independent samples Pre- and posttreatment values in the same group were compared by Student's paired *t*-tests. Differences in categorical variables were tested using chi-square tests. The relevance of the results was determined using a 95% confidence interval. A *t* statistic and two sample means were used to generate an interval estimate of the difference between two population means. The strength of the association between two variables was measured using Pearson's

[1] Assuming a significance level of 0.05, as well as a power of 80%, at least 15 men had to be included in each group of patients to detect a 20% difference in serum titers of thyroid antibodies.

correlation coefficient (r). Differences were reported as statistically significant if 95% confidence intervals did not include the null value and/or two-tailed p values were less than 0.05.

Results

There were no significant differences between both groups of men in age, body mass index, smoking habits, serum levels of thyrotropin, free thyroxine, free triiodothyronine and 25-hydroxyvitamin D levels, titers of TPOAb and TgAb, as well as in values of Jostel's thyrotropin index, SPINA-GD and SPINA-GT. Expectedly, testosterone-treated and testosterone-naïve men differed in serum levels of total testosterone (Table 1).

No serious adverse events were reported and all participants completed the study.

In both study groups, vitamin D increased 25-hydroxyvitamin D levels and SPINA-GT, as well as reduced TPOAb and TgAb titers. Moreover, in testosterone-treated men, vitamin D increased testosterone levels. The impact on antibody titers, SPINA-GT, testosterone and 25-hydroxyvitamin D was stronger in testosterone-treated than testosterone-naïve men. Neither in testosterone-treated nor in testosterone-naïve men, vitamin D affected circulating levels of thyrotropin, free thyroxine, free triiodothyronine and testosterone, as well as Jostel's thyrotropin index and SPINA-GD. Post-treatment values of thyroid antibody titers, SPINA-GT, testosterone and 25-hydroxyvitamin D differed between the study groups (Table 2).

At entry, there were correlations: between TPOAb and TgAb titers (vitamin D/testosterone: r = 0.59, $p < 0.001$; vitamin D: r=0.55, $p < 0.001$), between TPOAb and SPINA-GT (vitamin D/testosterone: r=−0.37, $p < 0.001$; vitamin D: r=−0.32, $p < 0.05$), between TPOAb and 25-hydroxyvitamin D levels (vitamin D/testosterone: r=−0.35, $p < 0.01$; vitamin D: r=−0.38, $p < 0.001$), between TgAb and SPINA-GT (vitamin D/testosterone: r=−0.25 $p < 0.05$; vitamin D: r=−0.30, $p < 0.05$), as well as between TgAb and 25-hydroxyvitamin D levels (vitamin D/testosterone: r=−0.30, $p < 0.05$; vitamin D: r=−0.28, $p < 0.05$). The effect of vitamin D on TPOAb titers correlated with: baseline TPOAb titers (vitamin D/testosterone: r=0.44, $p < 0.001$; vitamin D: r=0.48, $p < 0.001$), baseline TgAb titers (vitamin D/testosterone: r=0.38, $p < 0.001$; vitamin D: r=0.34, $p < 0.01$), baseline 25-hydroxyvitamin D levels (vitamin D/testosterone: r=−0.37, $p < 0.01$; vitamin D: r=−0.41, $p < 0.001$), treatment-induced changes in TgAb titers (vitamin D/testosterone: r=0.44, $p < 0.001$; vitamin D: r=0.48, $p < 0.001$), treatment-induced changes in 25-hydroxyvitamin levels (vitamin D/testosterone: r=0.32, $p < 0.05$; vitamin D: r=0.38, $p < 0.001$), as well as with treatment-induced changes in SPINA-GT (vitamin D/testosterone: r=0.29, $p < 0.05$; vitamin D: r=0.28, $p < 0.05$). In turn, the effect of vitamin D on TgAb titers correlated with: baseline TPOAb titers (vitamin D/testosterone: r=0.35, $p < 0.01$; vitamin D: r=0.31, $p < 0.05$), baseline TgAb titers (vitamin D/testosterone: r=0.40, $p < 0.001$; vitamin D: r=0.42, $p < 0.001$), baseline 25-hydroxyvitamin D levels (vitamin D/testosterone: r=−0.34, $p < 0.01$; vitamin D: r=−0.38, $p < 0.001$), treatment-induced changes in 25-hydroxyvitamin levels (vitamin D/testosterone: r=0.28, $p < 0.05$; vitamin D: r=0.29, $p < 0.05$), as well as with treatment-induced changes in SPINA-GT (vitamin D/testosterone: r=0.25, $p < 0.05$; vitamin D: r=0.27, $p < 0.05$). Moreover, in testosterone-treated patients, the effect of vitamin D on testosterone levels correlated with its impact on antibody titers (TPOAb: r=0.38, $p < 0.001$; TgAb: r=0.34, $p < 0.01$) and 25-hydroxyvitamin D levels (r=0.29, $p < 0.05$).

Discussion

Compared with other available modalities of testosterone replacement, the impact of oral testosterone undecanoate on serum testosterone levels is less prominent and is characterized by marked fluctuations [23]. However, over 80% of compliant hypogonadal men receiving oral testosterone undecanoate at the daily dose ranging from 120 to 240 mg show circulating testosterone levels within the reference range [24]. This suggests that the participants of the current study were characterized by a high degree of compliance with the treatment regimen.

In line with the results of our previous studies including women with autoimmune thyroid disorders [11,12], vitamin D therapy reduced serum levels of thyroid antibodies also in men with Hashimoto's thyroiditis. More importantly, this effect was stronger in individuals with late-onset hypogonadism receiving oral testosterone preparations than in testosterone-naïve men. This finding indicates that testosterone levels may determine the strength of vitamin D action on thyroid autoimmunity. Although the magnitude of the reduction in TPOAb and TgAb correlated with their baseline titers and well as with baseline 25-hydroxyvitamin D levels, similar pretreatment values of TPOAb, TgAb and 25-hydroxyvitamin D, being a consequence of the preselection procedure, indicate that the obtained results cannot be explained by various severity of thyroiditis in both study groups. However,

Table 1
Baseline characteristics of patients.

Variable	Testosterone-treated men	Testosterone-naive men	Difference [95% CI]
Number of patients	17	19	–
Age [years; mean (SD)]	57 (8)	59 (7)	2 [−3, 7]
Smokers [%]	29	32	–
Body mass index [kg/m^2; mean (SD)]	27.2 (4.4)	28.3 (4.6)	1.1 [−2.0, 4.2]
TPOAb [IU/mL; mean (SD)]	867 (287)	894 (256)	27 [−157, 211]
TgAb [IU/mL; mean (SD)]	810 (345)	792 (304)	−18 [−274, 238]
Thyrotropin [mIU/L; mean (SD)]	2.4 (1.1)	2.5 (1.0)	0.1 [-0.6, 0.8]
Free thyroxine [pmol/L; mean (SD)]	14.6 (2.3)	14.3 (2.1)	−0.3 [−1.8, 1.2]
Free triiodothyronine [pmol/L; mean (SD)]	3.2 (0.6)	3.2 (0.5)	0.0 [−0.4, 0.4]
Jostel's thyrotropin index [mean (SD)]	2.8 (0.3)	2.8 (0.3)	0.0 [−0.2, 0.2]
SPINA-GT index [pmol/s; mean (SD)]	2.38 (0.34)	2.28 (0.30)	−0.10 [−0.32, 0.12]
SPINA-GD index [nmol/s; mean (SD)]	20.27 (2.76)	20.69 (2.46)	0.42 [−1.35, 2.19]
Testosterone [ng/mL; mean (SD)]	5.3 (1.0)	2.2 (0.5)	−3.1 [−3.6, −2.6]*
25-hydroxyvitamin D [ng/mL; mean (SD)]	22.7 (5.8)	24.2 (6.1)	1.5 [−2.5, 5.5]

CI: confidence interval; IU: international unit; SD: standard deviation; SPINA: structure parameter inference approach; TgAb: thyroglobulin antibodies; TPOAb: thyroid peroxidase antibodies Table 2. The effect of vitamin D on thyroid antibody titers, hormones and thyroid function in testosterone-treated and testosterone-naïve euthyroid men with Hashimoto's thyroiditis and testosterone deficiency.

* Statistically significant difference between both groups.

Table 2
The effect of vitamin D on thyroid antibody titers, hormones and thyroid function in testosterone-treated and testosterone-naïve euthyroid men with Hashimoto's thyroiditis and testosterone deficiency.

	Testosterone-treated men	Testosterone-naive men	Difference [95% CI]
TPOAb [IU/mL; mean (SD)]			
Baseline	867 (287)	894 (256)	27 [−157, 211]
After 26 weeks	585 (186)[#]	724 (204)[#]	139 [6, 272][*]
Change	−282 (104)	−170 (84)	112 [48, 176][&]
TgAb [IU/mL; mean (SD)]			
Baseline	810 (345)	792 (304)	−18 [-274, 238]
After 26 weeks	527 (219)[#]	618 (202)[#]	91 [−52, 234]
Change	−283 (141)	−174 (84)	109 [43, 175][&]
Thyrotropin [mIU/L; mean (SD)]			
Baseline	2.4 (1.1)	2.5 (1.0)	0.1 [−0.6, 0.8]
After 26 weeks	2.1 (0.9)	2.4 (1.0)	0.3 [−0.3, 0.9]
Change	−0.3 (0.2)	−0.1 (0.2)	0.2 [−0.1, 0.5]
Free thyroxine [pmol/L; mean (SD)]			
Baseline	14.6 (2.3)	14.3 (2.1)	−0.3 [−1.8, 1.2]
After 26 weeks	15.9 (2.5)	15.3 (1.9)	−0.6 [−2.1, 0.9]
Change	1.3 (0.7)	1.0 (0.6)	−0.3 [−0.8, 1.4]
Free triiodothyronine [pmol/L; mean (SD)]			
Baseline	3.2 (0.6)	3.2 (0.5)	0.0 [−0.4, 0.4]
After 26 weeks	3.5 (0.7)	3.4 (0.6)	−0.1 [−0.5, 0.3]
Change	0.3 (0.2)	0.2 (0.2)	−0.1 [−0.3, 0.1]
Jostel's thyrotropin index [mean (SD)]			
Baseline	2.8 (0.3)	2.8 (0.3)	[−0.2, 0.2]
After 26 weeks	2.9 (0.2)	2.9 (0.2)	[−0.1, 0.1]
Change	0.1 (0.1)	0.1 (0.1)	0.0 [−0.1, 0.1]
SPINA-GT index [pmol/s; mean (SD)]			
Baseline	2.38 (0.34)	2.28 (0.30)	−0.10 [−0.32, 0.12]
After 26 weeks	2.79 (2.28)	2.49 (0.32)	−0.30 [−0.51, −0.09][*]
Change	0.41 (0.20)	0.21 (0.16)	−0.20 [−0.32, −0.08][&]
SPINA-GD index [nmol/s; mean (SD)]			
Baseline	20.27 (2.76)	20.69 (2.46)	0.42 [−1.35, 2.19]
After 26 weeks	20.35 (3.05)[#]	20.55 (2.09)[#]	0.20 [−1.55, 1.95]
Change	0.08 (0.25)	−0.14 (0.43)	−0.22 [−0.46, 0.02]
Testosterone [ng/mL; mean (SD)]			
Baseline	5.3 (1.0)	2.2 (0.5)	−3.1 [−3.6, −2.6][*]
After 26 weeks	6.1 (1.2)[#]	2.3 (0.4)	−3.8 [−4.4, −3.2][*]
Change	0.8 (0.4)	0.1 (0.2)	−0.7 [−0.9, −0.5][&]
25-hydroxyvitamin D [ng/mL; mean (SD)]			
Baseline	22.7 (5.8)	24.2 (6.1)	1.5 [−2.5, 5.5]
After 26 weeks	41.9 (6.0)[#]	36.0 (5.5)[#]	−5.9 [−9.8, −2.0][*]
Change	19.2 (4.6)	11.8 (3.2)	−7.4 [−10.1, −4.7][&]

CI: confidence interval; IU: international unit; SD: standard deviation; SPINA: structure parameter inference approach; TgAb: thyroglobulin antibodies; TPOAb: thyroid peroxidase antibodies.
[*] Statistically significant difference between both groups.
[#] Statistically significant difference between post-treatment and baseline values in the same group.
[&] Statistically significant difference between the changes in both groups.

the presence of these correlations suggests that men with more advanced stages of thyroid autoimmune disease, as well as subjects with severe vitamin D deficiency may benefit more from cholecalciferol administration than men with normal or mildly disturbed vitamin D status.

Both systemic and organ-specific autoimmune disorders cannot be cured and are difficult to treat. However, testosterone therapy of men with testosterone deficiency, by regulating multiple and overlapping cellular and molecular pathways involving immune cells and biochemical factors, may bring clinical benefits to men with numerous autoimmune disorders, including rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, psoriasis and multiple sclerosis [25,26]. The obtained results indicate that Hashimoto's thyroiditis is another autoimmune disorder, the clinical course of which may be slowed down by this hormone and that its use may be justified in hypogonadal men in whom autoimmune thyroiditis coexists with other autoimmune disorder. Moreover, taking into account synergistic effects on antibody titers of testosterone and exogenous vitamin D, being another factor suppressing the development of autoimmunity [27], testosterone/vitamin D combination therapy may be recommended particularly in men at the highest risk of progression to hypothyroidism.

The effect on antibody titers was paralleled by an increase in SPINA-GT. Compared to healthy subjects, patients with autoimmune thyroiditis were characterized by lower values of SPINA-GT, even if their thyrotropin and free thyroid levels were within the reference range [28]. Moreover, SPINA-GT was found to show less intra-individual variation than circulating levels of thyrotropin, free thyroxine and free triiodothyronine [21,22]. Based on these studies, it may be assumed that even a moderate destruction of the thyroid gland secondary to lymphocytic infiltration and fibrosis is reflected by a decrease in SPINA-GT. In turn, vitamin D-induced increase in SPINA-GT indicates that a beneficial effect of vitamin D on thyroid autoimmunity leads to the improvement in secretory capacity of the thyroid gland, as well as that this improvement is more pronounced in men with compensated testosterone deficiency. Unlike the maximum secretory capacity of the thyroid gland, irrespective of testosterone supplementation, vitamin D therapy does not seem to affect pituitary thyrotropic function and the conversion rate of thyroxine to triiodothyronine. In line with this explanation, neither in testosterone-treated and testosterone-

naïve men, exogenous cholecalciferol exerted a significant effect on Jostel's thyrotropin index [20], being a quantitative marker for pituitary thyrotropic function, as well as on SPINA-GD, measuring the efficiency of thyroid de-iodination at the level of peripheral tissues [21,22].

Further research is required to explain the molecular mechanisms underlying our findings. Recently, we have observed that testosterone undecanoate reduces thyroid antibody titers in drug-naïve men with Hashimoto's thyroiditis and low testosterone levels, as well as that this effect was strongest after 6 months of therapy [Krysiak et al., unpublished]. Similar baseline antibody titers in the present study may be easily explained by the preselection procedure aimed at obtaining two groups of patients with Hashimoto's thyroiditis of similar intensity. Because, the participants had received testosterone for at least 6 months before the beginning of the present study, the strength of testosterone action does not seem to have increased with time. However, the more pronounced effect on thyroid autoimmunity and thyroid secretory function may be partially attributed to an increase in testosterone levels observed in the arm receiving both testosterone undecanoate and vitamin D. Although in a human testicular cell culture model, cholecalciferol increased testosterone production [29], no changes in testosterone levels were observed in a clinical study including men with testicular failure [30]. In line with this finding, testosterone levels in testosterone-naïve men remained at the similar level throughout the current study. Therefore, more probable is that vitamin D increased testosterone levels in subjects receiving testosterone therapy by affecting the pharmacokinetics of exogenous testosterone undecanoate rather than by stimulating endogenous testosterone production. Theoretically, vitamin D may increase sex hormone-binding globulin production, may reduce hepatic testosterone inactivation, may inhibit renal testosterone excretion, as well as may decrease testosterone conversion to 5α-dihydrotestosterone or estradiol.

Differences in the strength of action of exogenous cholecalciferol on thyroid autoimmunity between both study groups may be also partially explained by the impact of testosterone on vitamin D metabolism. In line with this hypothesis, the effect of vitamin D plus testosterone on antibody titers and 25-hydroxyvitamin D levels correlated with treatment-induced changes in testosterone levels. There are some arguments indicating that testosterone may affect vitamin D homeostasis. Men who had undergone unilateral testicular orchidectomy were characterized by lower 25-hydroxyvitamin D levels than matched healthy controls and these differences were attributed to a decrease in the activity of cytochrome P450 2R1, an enzyme playing a key role in 25-hydroxylation of vitamin D [31]. Five-year intramuscular treatment with testosterone undecanoate of aging men with metabolic syndrome [32], as well as testosterone-replacement therapy of bilaterally orchidectomized men [33] increased circulating levels of 25-hydroxyvitamin D levels. Puberty progression was accompanied by an increase in 25-hydroxyvitamin D levels [34]. Orchidectomy resulted in a reduction of serum 25-hydroxyvitamin D and calcitriol levels in rats and these changes were corrected by testosterone replacement [35]. On the basis of these findings, it seems likely that the effective conversion of cholecalciferol to 25-hydroxyvitamin D depends on the availability of sufficient amounts of testosterone in Leydig cells [36] and possibly also in other cells in which this conversion takes place. In subjects with low testosterone levels this conversion may be suboptimal and therefore the strength of vitamin D action is less pronounced.

The obtained results should be interpreted in the light of some study limitations. The main drawback of the study is a small sample size and its non-randomized design. It cannot be also totally excluded that the effect of vitamin D would be different in men using other forms of testosterone replacement therapy (transdermal patches, topical gels, intramuscular preparations, topical solutions and buccal tablets). Moreover, it cannot be ruled out that the effect of cholecalciferol does not have to be the same in testosterone-treated patients in whom baseline testosterone levels were within the reference range. Finally, because the study included men with sufficient iodine [37] and inadequate selenium [38] intake, it is uncertain whether vitamin D exerts a similar effect in patients living in iodine-deficient and/or selenium-adequate areas.

In conclusion, 26-week treatment with vitamin D decreased TPOAb and TgAb titers, as well as increased SPINA-GT in euthyroid men with Hashimoto's thyroiditis. These effects were stronger in testosterone-treated than testosterone-naïve subjects, as well as correlated with baseline antibody titers and baseline 25-hydroxyvitamin D levels. The obtained results indicate that the impact of cholecalciferol on thyroid autoimmunity and thyroid function is fully expressed only if testosterone deficiency is compensated. Larger and multicenter clinical trials are required to confirm our early findings.

Disclosure statement

The authors declare no conflicts of interest

Author contributions

Robert Krysiak conceived of the study, participated in its design, performed the statistical analysis, as well as drafted and edited the manuscript. Karolina Kowalcze conducted the literature search and performed the statistical analysis. Bogusław Okopień participated in its design and coordination, and provided critical input during manuscript preparations. All authors read and approved the final manuscript.

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